Ecol. *54*, 300–314.
3. Tarling, G.A., and Johnson, M.L. (2006). Satiation gives krill that sinking feeling. Curr. Biol. *16*, R83–R84.
4. Leising, A.W., Pierson, J.J., Cary, S., and Frost, B.W. (2005). Copepod foraging and predation within the surface layer during night-time feeding forays. J. Plankt. Res. *27*, 987–1001.
5. Beaumont, K.L., Nash, G.V., and Davidson, A.T. (2002). Ultrastructure, morphology and flux of microzooplankton faecal pellets in an east Antarctic fjord. Mar. Ecol. Prog. Ser. *245*, 133–148.
6. Cadée, G.C., González, H., and Schnack-Schiel, S.B. (1992). Krill diet affects faecal string settling. Polar Biol. *12*, 75–80.
7. Broecker, W.S., and Peng, T.H. (1982). Tracers in the Sea. Lamont Doherty Geological Observatory, Columbia University, 690 pp.
8. Suzuki, H., Sasaki, H., and Fukuchi, M. (2003). Loss processes of sinking fecal pellets of zooplankton in the mesopelagic layers of the Antarctic marginal ice zone. J. Oceanogr. *59*, 809–818.
9. Huntley, M.E., and Zhou, M. (2004). Influence of animals in turbulence in the sea. Mar. Ecol. Prog. Ser. *273*, 65–79.
10. Godlewska, M. (1996). Vertical migrations of krill (*Euphausia superba* Dana). Pol. Arch. Hydrobiol. *43*, 9–63.
11. Zhou, M., and Dorland, R.D. (2004). Aggregation and vertical migration behavior of *Euphausia superba.* Deep-sea Res. II *51*, 2119–2137.

Tasmanian Aquaculture and Fisheries Institute and School of Zoology, University of Tasmania, Private Bag 49, Hobart, Tasmania 7001, Australia.

DOI: 10.1016/j.cub.2006.02.044

Stem Cells: Specifying Stem-Cell Niches in the Worm

Recent work has shown that components of the Wnt signaling pathway directly activate a homeodomain transcription factor so as to specify the cell fate that provides niche function to germline stem cells in the nematode *Caenorhabditis elegans*.

Samantha Van Hoffelen and Michael A. Herman

The location, identity, fate and control of stem cell populations are active areas of research spanning many disciplines of biology. Stem cells, whether embryonic, adult, somatic or germ-line precursors reside in specific compartments known as 'stem cell niches' [1,2]. The concept of the 'niche' is far from new, however, the molecular characterization of such microenvironments is still very much under study. Lam *et al*. [3] have recently reported work which sheds new light on the signaling pathway leading to specification of the germline stem-cell niche in the nematode *Caenorhabditis elegans*.

Stem-cell niches are defined by their ability to maintain proliferative pools of cells. By secreting factors and providing an organized architecture, niches maintain cells in mitosis while promoting self-renewal. Niches have been described in many tissues, including the hematopoietic system, skin, intestinal epithelium, neural tissue and reproductive system in animals, and roots in plants [4].

Cell–cell adhesion between niche cells and stem cells, accompanied by secreted niche factors, control the fate of stem cells while allowing cells that migrate from the niche to differentiate (or undergo meiosis in the case of the germline). In the *Drosophila* testis, germline stem cells form a ring around differentiated cells known as the hub [5]. Upd signals from hub cells activate the JAK/STAT pathway, causing germline stem cells to divide, each giving rise to a germline stem cell and a gonialblast, which subsequently differentiates. Only the cells adjacent to the hub receive Upd signals and undergo self-renewal; so that the hub cells provide the niche for the *Drosophila* male germline [6].

Other systems are not so well defined and the exact identity, much less the specification, of their niche cells is not well characterized. In *C. elegans*, the distal tip cell and its extended processes provide a niche for proliferating germ cells. Although it is not yet clear how stem-like the *C. elegans* germline stem cells really are, it is clear that they remain undifferentiated in the distal tip cell niche and give rise to differentiated progeny; giving them stem-like qualities that are worthy of study.

C. elegans exists as either hermaphrodite or male sexes. In a hermaphrodite, the distal tip cells lie at the end of each arm of the U-shaped gonad (Figure 1A). In a male, there is one distal tip cell at the end of their single-armed gonad. The adult hermaphrodite germline has a 'mitotic region' at the distal end of the gonad and a more proximal 'transition zone'. Germline stem cells undergo self-renewal in the mitotic region, which extends about 20 cell diameters along the distal-proximal axis. Just beyond that lies the transition zone, where the germline nuclei begin differentiating and start to enter the early stages of meiotic prophase [7] (Figure 1B).

The distal tip cell and its processes maintain contact with germline stem cells in the mitotic region and express LAG-2, a Delta-like ligand, which binds to GLP-1, a Notch-like receptor expressed by the germline stem cells. GLP-1/Notch signaling activates the Pumilio family RNA binding proteins FBF-1 and FBF-2. These proteins regulate the stability of the *gld-1* mRNA that encodes an RNA binding protein that represses the translation of factors required to maintain mitosis, like GLP-1 [7].

Regulation of mRNA stability or translation within germline stem cells is conserved in *C. elegans* and *Drosophila* germlines [6]. Removal of the distal tip cell, or loss-of-function of *lag-2* or *glp-1*, causes the germline stem cells to enter meiosis prematurely, thereby loosing their stem-cell identity. Conversely, constitutive GLP-1 signaling blocks entry into meiosis and causes overproliferation of the germ cells. The distal tip cell (and its processes) provides an environment that is required for

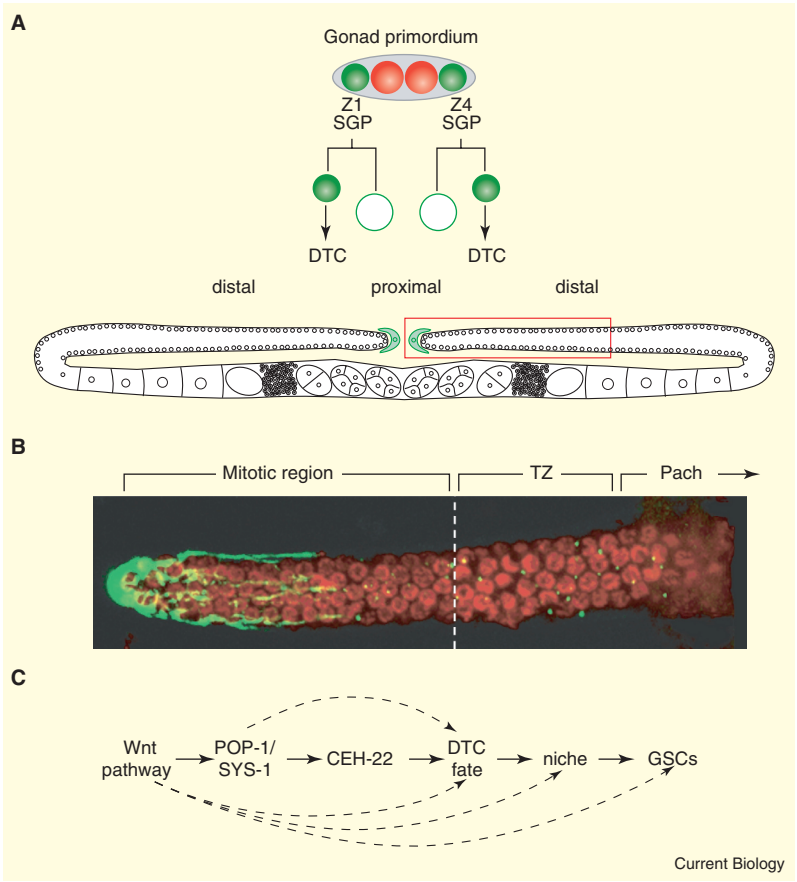

Figure 1. Wnt-controlled asymmetric cell divisions generate the distal tip cell that provides a niche function to the germline stem cells.

(A) The *C. elegans* gonad primordium consists of four cells, Z1–Z4. The distal cells (green) are the somatic gonad precursors and give rise to the gonad, while the inner cells (red) give rise to the germline. The somatic gonad precursors each divide asymmetrically leading to the generation of the distal tip cells (DTCs) that provide leader function during morphogenesis of the U-shaped hermaphrodite gonad and niche function for the germline stem cells in adults. The boxed region is shown in detail in (B). (B) Fluorescent micrograph of the distal arm of the hermaphrodite gonad. The distal tip cell and its processes are visualized using GFP (green), produced under control of the *lag-2* promoter in a projection of a confocal z-series. Germline nuclei are visualized with ToPro-3 (red) in an adult hermaphrodite gonad dissected from the animal, a subset of sections of the confocal z-series projection are shown to make nuclear morphology clearer. Mitotic region, transition zone (TZ) and differentiated pachytene region (Pach) are marked with brackets. The distal to proximal axis of the germ line extends from the distal tip cell at the distal end to mature gametes at the proximal end. Reprinted with permission from [6]. (C) A Wnt pathway functions through POP-1/Tcf and SYS-1/β-catenin to directly control expression of CEH-22/Nkx2.5 that leads to the specification of distal tip cell fate, a role of which is to provide niche function to the germline stem cells. Solid arrows indicate known direct or indirect regulation of one component or process by another. Dashed arrows indicate other possible levels of regulation that have not yet been ruled out. For example, since the distal tip cells do not form in *pop-1* or *sys-1* mutants, a role for the Wnt pathway in germline stem-cell maintenance has not been able to be assessed.

germline stem-cell maintenance, thus it constitutes a stem-cell niche. Other niche-stem cell interactions that use Notch signaling are described in the hematopoietic system [8], nervous system [9] and skin [10].

How is a niche cell made? The distal tip cells arise through asymmetric divisions of the two somatic gonad precursor cells (Figure 1A). Each somatic gonad precursor cell divides along the proximal-distal axis and the distal daughters give rise to the distal tip cells. Mutations that disrupt the asymmetric somatic gonad precursor cell divisions cause a Sys — for symmetric sister — defect in which both somatic gonad precursor cell daughters take the proximal fate. Genetic screens for Sys mutants yielded mutations in *pop-1*/Tcf [11], *sys-1*/β-catenin [12, 13] and *sys-3* or *ceh-22*/ Nkx2.5 [3, 14]. WRM-1/β-catenin and LIT-1 kinase are also involved [14]. These are known components of the Wnt signaling pathway, which clearly controls the asymmetric division of the somatic gonad precursors cells and thus the fate of the niche cell; the specific Wnt ligand, however, has not yet been identified.

Wnts play central roles in many aspects of development; their roles in stem-cell maintenance and differentiation are well characterized for the hematopoietic and intestinal systems [15]. Wnts are secreted glycoproteins which bind to Frizzled transmembrane receptors. In the canonical pathway, such activation causes Disheveled to inactivate GSK3β which frees β-catenin from the 'destruction complex', allowing β-catenin to translocate to the nucleus where it complexes TCF/LEF family members to activate target genes. Along the crypt-villus axis of the small intestinal epithelium there is a transition from proliferation and self-renewal at the crypt base — 'the niche' — toward differentiation at the distal aspect of the villus. Wnt signaling in the niche maintains a proliferative phenotype with a defined Wnt gradient being the control between proliferating stem cells and differentiated epithelial cells [16]. Furthermore, inhibition of Wnt signaling induces the complete loss of crypts in adult mice. Hematopoietic stem cells reside in a niche partly created by osteoblasts. Wnt signaling has been shown to regulate the self-renewal and maintenance of hematopoietic stem cells, moreover, Wnts are secreted by the hematopoietic stem cells themselves. Wnts promote the proliferation and prevent the differentiation of hematopoietic stem cells within the niche. To date there has been no evidence to suggest Wnts both specify the

niche and regulate the stem cells within the niche.

Lam *et al*. [3] show that *ceh-22* is a direct target of a Wnt pathway controlling the asymmetric SGP division that generates the distal tip cell and thus the niche. Specifically, POP-1/Tcf and SYS-1/ β-catenin activate *ceh-22* expression in the distal somatic gonad precursor cell daughters via POP-1 binding sites in the *ceh-22* promoter. The expression of the homeodomain transcription factor Nkx2.5, a homolog of CEH-22, is also controlled by Wnt signaling, although it has not been shown to be a direct target [17]. Interestingly, POP-1 is also expressed asymmetrically in the somatic gonad precursor cell daughters, but its level is lower in the nucleus of the distal daughter than in the proximal daughter [14]. Thus *ceh-22* is activated in the cell with lower nuclear POP-1 levels in a manner recently proposed by Kidd *et al.* [13].

Finally, Lam *et al*. [3] showed that CEH-22 is sufficient for distal tip cell fate as *ceh-22* expressed from a heterologous heat-shock promoter can rescue *ceh-22* mutants and even cause both SGP daughters to become distal tip cells. Amazingly, these ectopic distal tip cells are able to provide niche function! Thus, distal tip cell fate, an aspect of which is to specify the niche, is controlled by CEH-22 [3].

The finding that Wnt specifies the distal tip cell niche is intriguing and it will be important to determine whether Wnt signaling also acts within the niche along with notch to promote mitosis. Although it is premature to say that CEH-22 or its homolog Nkx2.5 is a 'niche-specifying gene', they are likely to control, either directly or indirectly, the niche genes (Figure 1C). Further analysis of the distal tip cell niche and the role of CEH-22 in the control of distal tip cell fate described by Lam *et al.* [3] may lead to the identification of niche-specifying genes such as those that control cell adhesion and regulate production of niche signals. Study of the simplified *C. elegans* distal tip cell/germline stem-cell niche and stem-cell population is likely to yield further important discoveries.

References

1. Watt, F.M., and Hogan, B.L. (2000). Out of Eden: stem cells and their niches. Science *287*, 1427–1430.
2. Ohlstein, B., Kai, T., Decotto, E., and Spradling, A. (2004). The stem cell niche: theme and variations. Curr. Opin. Cell Biol. *16*, 693–699.
3. Lam, N., Chesney, M.A., and Kimble, J. (2006). Wnt signaling and CEH-22/tinman/Nkx2.5 specify a stem cell niche in C. elegans. Curr. Biol. *16*, 287–295.
4. Sabatini, S., Heidstra, R., Wildwater, M., and Scheres, B. (2003). SCARECROW is involved in positioning the stem cell niche in the *Arabidopsis* root meristem. Genes Dev. *17*, 354–358.
5. Yamashita, Y.M., Fuller, M.T., and Jones, D.L. (2005). Signaling in stem cell niches: lessons from the *Drosophila* germline. J. Cell Sci. *118*, 665–672.
6. Xie, T., Kawase, E., Kirilly, D., and Wong, M.D. (2005). Intimate relationships with their neighbors: tales of stem cells in *Drosophila* reproductive systems. Dev. Dyn. *232*, 775–790.
7. Kimble, J., and Crittenden, S.L. (2005). Germline proliferation and its control. (August *15*, 2005), Wormbook, ed., The C. elegans Research Community, Wormbook, doi/10.1895/wormbook.1.13.1, http://www.wormbook.org.
8. Reya, T., Duncan, A.W., Ailles, L., Domen, J., Scherer, D.C., Willert, K., Hintz, L., Nusse, R., and Weissman, I.L. (2003). A role for Wnt signalling in self-renewal of haematopoietic stem cells. Nature *423*, 409–414.
9. Hitoshi, S., Alexson, T., Tropepe, V., Donoviel, D., Elia, A.J., Nye, J.S., Conlon, R.A., Mak, T.W., Bernstein, A., and van der Kooy, D. (2002). Notch pathway molecules are essential for the maintenance, but not the generation, of mammalian neural stem cells. Genes Dev. *16*, 846–858.
10. Lowell, S., Jones, P., Le Roux, I., Dunne, J., and Watt, F.M. (2000). Stimulation of human epidermal differentiation by delta-notch signalling at the boundaries of stem-cell clusters. Curr. Biol. *10*, 491–500.
11. Siegfried, K.R., and Kimble, J. (2002). POP-1 controls axis formation during early gonadogenesis in *C. elegans.* Development *129*, 443–453.
12. Miskowski, J., Li, Y., and Kimble, J. (2001). The *sys-1* gene and sexual dimorphism during gonadogenesis in *Caenorhabditis elegans.* Dev. Biol. *230*, 61–73.
13. Kidd, A.R., 3rd, Miskowski, J.A., Siegfried, K.R., Sawa, H., and Kimble, J. (2005). A beta-catenin identified by functional rather than sequence criteria and its role in Wnt/MAPK signaling. Cell *121*, 761–772.
14. Siegfried, K.R., Kidd, A.R., 3rd, Chesney, M.A., and Kimble, J. (2004). The *sys-1* and sys-3 genes cooperate with Wnt signaling to establish the proximal-distal axis of the *Caenorhabditis elegans* gonad. Genetics *166*, 171–186.
15. Reya, T., and Clevers, H. (2005). Wnt signalling in stem cells and cancer. Nature *434*, 843–850.
16. Pinto, D., and Clevers, H. (2005). Wnt control of stem cells and differentiation in the intestinal epithelium. Exp. Cell Res. *306*, 357–363.
17. Terami, H., Hidaka, K., Katsumata, T., Iio, A., and Morisaki, T. (2004). Wnt11 facilitates embryonic stem cell differentiation to Nkx2.5-positive cardiomyocytes. Biochem. Biophys. Res. Commun. *325*, 968–975.

Program in Molecular Cellular and Developmental Biology, Division of Biology, Kansas State University, Manhattan, Kansas 66506-4901, USA.
E-mail: mherman@ksu.edu

DOI: 10.1016/j.cub.2006.02.043